Please add new claims 27 and 28 as follows:

27. (New) An assay of claim 1, wherein the substrate is made of an insoluble non-porous material.

C4

28. (New) An assay of claim 1, wherein a binding capacity of the microscopic sorbent zone of 150 μm in diameter is about 10^{10} analyte molecules.

REMARKS:

Claims 1, 2, 23, and 26 are amended. New claims 27-28 are added. No new matter is introduced. Claims 1-28 are pending in the application. Reexamination and reconsideration of the application, as amended, are respectfully requested.

Claims 1-4, 13-19, 21, and 23-26 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Ekins *et al.,* EP 304,202, (the '202 patent) in view of Ekins *et al.*, J. of Clinical Immunoassay (Immunoassay reference). This rejection is respectfully traversed.

The present invention relates to a microscale binding assay, an analyte binding array and a kit for use in a binding assay with a high sensitivity for very low quantities of analyte. The invention teaches an array of microscopic sorbent zones with a high concentration of binding partner per unit area of a sorbent zone. As explained on pages 9-10 of the instant specification, the microscopic sorbent zones are discrete regions on the substrate with a diameter of less than 500 μm. The gist of the present invention is an array of microscopic sorbent zones formed by confining an excess of analyte binding partner (relative to the total amount of analyte present in the sample) to microscopic areas of a substrate to achieve high density of the analyte binding partner. The microscopic sorbent zones of the present invention unexpectedly deplete substantially all analyte from the sample and concentrate the analyte onto the small measurement region, thus, serving as analyte concentrating devices. (p. 7, lines 21-26). As a result, the analyte signal and the signal-to-background ratio greatly increases during image detection.

Accordingly, the independent claims 1, 23, and 26 have been amended to emphasize three crucial features of the present invention: microscopic size of the sorbent zones, substantial depletion of analyte from the sample, and concentration of the depleted analyte on the microscopic sorbent zones. As explained on page 14, lines 17-22, of the instant specification,

"substantial depletion" means that "at least about 60% of the analyte will be captured by a high affinity binding partner" (emphasis added).

The Examiner acknowledged that the '202 patent does not teach the analyte being substantially depleted from the sample, but relied on the Immunoassay reference for teaching the same. Applicants respectfully disagree with the Examiner's reading of the Immunoassay reference.

The Examiner notes that the Immunoassay reference teaches "analyte depletion in the surrounding medium." Analyte depletion in the surrounding medium, however, does not constitute substantial depletion of any analyte present in the defined sample volume as required by claims 1, 23, and 26. As explained on page 7, lines 21-31, in the present invention, the analyte depletion is so substantial that it maximally perturbs bulk concentration of analyte in the sample. As a result, the developed signal reflects total analyte mass in the defined sample volume. The Immunoassay reference, on the contrary, teaches only an insignificant and localized analyte depletion in the medium immediately surrounding analyte binding sites. Such minuscule analyte depletion does not appreciably affect the overall bulk concentration of the analyte in the sample solution (page 173, left column). Accordingly, the developed signal is indicative of ambient analyte concentration, but not the total analyte mass in the sample.

Additionally, neither the '202 patent nor the Immunoassay reference teaches concentrating analyte on the microscopic sorption zone as required by claims 1, 23, and 26. On the contrary, both references require that "only an insignificant proportion of any analyte present in the liquid sample becomes bound to the binding agent" (claim 1 of the '202 patent, emphasis added) and "[t]he percentage binding of analyte to antibody ... is <1%" (the Immunoassay reference, Fig. 4 legend). Accordingly, the sorbent zone of the '202 patent and the Immunoassay reference are not capable of concentrating the analyte. Because Ekins' assays fail to measure 99% of the available analyte in the sample, they generate a fluorescent signal that is from 60 to 100 times weaker than the signal produced in assay of the present invention (page 8, lines 10-23, and Figure 1).

Neither the '202 patent nor the Immunoassay reference suggest concentrating the analyte on the microscopic sorption zone as required by amended claims 1, 23, and 26. In order to facilitate the maximum harvest of the analyte and to concentrate the analyte on the microscopic sorbent zones, the instant application calls for minimization of the sample volume (page 8, lines

29-30) and maximization of the amount of analyte binding partner immobilized within sorbent zones (page 10, lines 30-33). The Immunoassay reference, in contrast, emphasizes that in the ambient analyte immunoassay, the analyte concentration in the medium is independent both of sample volume and of the amount of binding partner in the system (p. 172, right column – 173, left column).

Thus, the '202 patent and the Immunoassay reference, either alone or in combination, do not teach or suggest the analyte be substantially depleted from the bulk sample solution and concentrated on a microscopic sorbent zone. To the contrary, they teach away from the present invention by requiring that the analyte is depleted only locally and only an insignificant amount of the analyte (less than 1%) is accumulated on the sorbent zones. In fact, if a substantial amount of analyte is depleted from the sample bulk and concentrated on the sorbent zones, as required by the present invention, "the proportional occupancy of binding sites" required by Ekins would not be satisfied due to saturation of the binding sites. As a result, the Ekins assay would not respond to the analyte concentration in the sample.

The Examiner also appears to believe that "the technique of allowing the analyte depletion in a sample ... is already well known in the art" and can be incorporated into the binding assay of the '202 patent. Applicants respectfully disagree.

The present invention provides an unexpected benefit of high signal-to-background ratio of binding assay by concentrating the signal on the small area of the support. This benefit is derived from confinement of the binding partner to small area of support resulting in analyte concentration, rather than from simply providing large amounts of the binding partner to deplete analyte from the solution. In fact, another unanticipated benefit of the instant invention is reduction by at least 100-fold of the overall amount of the binding partner (p. 23, lines 25-32) as compared to the conventional methods. For example, as explained on p. 20, lines 3-6, this benefit is achieved by increasing the binding partner density on the sorbent zones by about 20 times over conventionally used levels.

Although it is true that methods such as affinity chromatography do deplete proteins from the solution by specific binding, the conventional methods do not concentrate the analyte onto a microscopic area. It is an unexpected discovery of the present invention that microscopic sorbent zones can substantially deplete analyte from a macroscopic (~ 100 μl) sample volume

and, therefore, substantially reduce bulk concentration of the analyte in the sample solution (p.7, lines 30-31 and p.21, lines 3-6).

In light of the foregoing, applicants respectfully submit that the '202 patent and the Immunoassay reference, either alone or in combination with other known techniques of the art, cannot make claims 1, 23, and 26 obvious. None of the cited references, either alone or in combination, would have motivated one skilled in the art to arrive at the present invention, which requires a substantial depletion of the analyte from the bulk solution and concentration of the analyte on the microscopic sorbent zones. Claims 2-4, 13-19, 24, and 25 depend, directly or indirectly, on claims 1 and 23 and are not obvious for at least the same reasons. Accordingly, withdrawal of the rejection to claims 1-4, 13-19, and 23-26 is respectfully requested.

Claims 1-4, 13-19, 21, and 23-26 are rejected under 35 U.S.C. § 103(a) as being unpatentable over the '202 patent in view of Ekins *et al.*, Analytica Chimica Acta (Analytica reference). This rejection is respectfully traversed.

As discussed above, the '202 patent does not teach an assay that requires a substantial depletion of the analyte from a sample and concentration of the analyte on the microscopic sorbent zones. The Analytica reference cannot remedy the defect of the '202 patent. As discussed in our previous response to the first Office Action, similarly to the Immunoassay reference, the Analytica reference discloses an ambient analyte immunoassay. It is required by the method that the proportion of bound analyte be so small that the "disturbance to the ambient analyte concentration can be ignored" (page 80, first paragraph). This "ignored" amount of the analyte depleted from the sample is further defined as "invariably less than 1% regardless of the analyte concentration" by the reference (page 80, first paragraph, last sentence). Therefore, like the Immunoassay reference, the Analytica reference does not teach or suggest the analyte being substantially depleted from the sample and concentrated on the sorbent zones. Instead, the Analytica reference teaches away from the present invention by requiring that only an insignificant amount of the analyte (less than 1%) is depleted from the sample. In light of the foregoing, applicants respectfully submit that the '202 patent and the Analytica reference, either alone or in combination, cannot make claims 1, 23, and 26 obvious. Claims 2-4, 13-19, 24, and 25 depend, directly or indirectly, on claims 1 and 23 and are not obvious for at least the same reasons. Accordingly, withdrawal of the rejection to claims 1-4, 13-19, 21, and 23-26 is respectfully requested.

Claims 5-10 are rejected under 35 U.S.C. § 103(a) as being unpatentable over the '202 patent, and either the Immunoassay reference or the Analytica reference, in further view of Ullman *et al.* (U.S. Patent 5,512,659). Claim 11 is rejected under 35 U.S.C. § 103(a) as unpatentable over the '202 patent, and either the Immunoassay reference or the Analytica reference in further view of Waggoner *et al.* (U.S. Patent 5,368,486). Claim 12 is rejected under 35 U.S.C. § 103(a) as being unpatentable over the '202 patent, and either the Immunoassay reference or the Analytica reference in view of the Waggoner *et al.*, in further view of Lee *et al.* (U.S. Patent 5,453,505). Claim 20 is rejected under 35 U.S.C. § 103(a) as being unpatentable over the '202 patent, and either the Immunoassay reference or the Analytica reference in view of Northrup *et al.* (U.S. Patent 5,639,423). Applicants respectfully traverse these rejections.

As discussed above, the '202 patent, the Immunoassay reference, and the Analytica reference, either alone or in combination, cannot make claim 1 obvious, because they teach away from the binding assay of the present application, which requires the analyte to be substantially depleted from the sample and concentrated on microscopic sorbent zones. Claims 5-10, 11, 12, and 20 depend directly or indirectly from claim 1 and cannot be made obvious by the '202 patent, the Immunoassay reference, and the Analytica reference for at least the same reasons.

Ullman *et al.*, Waggoner *et al.*, Lee *et al.*, and Northrup *et al.* cannot remedy the defect of the '202 patent, the Immunoassay reference, and the Analytica reference, and are not relied upon by the Examiner for such. Ullman *et al.*, Waggoner *et al.*, Lee *et al.*, and Northrup *et al.* have no teaching whatsoever of a binding assay utilizing a plurality of sorbent zones containing an analyte binding partner, let alone a binding assay, which requires an excess of the analyte binding partner relative to the analyte, so that any analyte present is substantially depleted from the sample and concentrated in the sorbent zones. Therefore, none of the cited references, either alone or in combination, can motivate one skilled in the art to arrive at claims 5-10, 11, 12, and 20. Withdrawal of the rejection is, therefore, respectfully requested.

Applicant has added new claims 27-28 to further define the scope of the invention. Support for new claim 27 can be found on pages 9, lines 14-15 of the application. Support for new claim 28 can be found on pages 8, lines 2-7 of the application. Claims 27-28 depend from claim 1 and are, therefore, believed to be patentable for at least the same reasons as claim 1.

In view of the foregoing, it is respectfully submitted that the application is in condition for allowance. Reexamination and reconsideration of the application, as amended, are requested.

If for any reason the Examiner finds the application other than in condition for allowance, the Examiner is requested to call the undersigned attorney at the Los Angeles, California, telephone number 213-337-6700 to discuss the steps necessary for placing the application in condition for allowance.

Respectfully submitted,

HOGAN & HARTSON, LLP

Date: May 17, 2001

By: _____

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Version with markings to show changes made:

1. (Amended) A binding assay for sensing analyte mass in a liquid sample, comprising:

a) immobilizing an array on a substrate, wherein the array comprises a plurality of microscopic sorbent zones, wherein a microscopic sorbent zone comprises an analyte binding partner,

b) contacting a defined volume of sample believed to contain an analyte with at least one microscopic sorbent zone, the analyte binding partner in the microscopic sorbent zone being present in excess relative to the analyte, so that any analyte present in the defined volume is substantially depleted from the sample and concentrated on the microscopic sorbent zone to form an analyte capture complex with the analyte binding partner;

c) tagging the analyte capture complex with a fluorescent label;

d) illuminating the microscopic sorbent zone with a laser in the absence of liquid;

e) detecting fluorescence emissions from any microscopic sorbent zone having an analyte capture complex tagged with a fluorescent label, thereby determining the analyte mass harvested from the defined volume of sample.

2. (Amended) An assay according to claim [1] 27 wherein the substrate is selected from the group consisting of polycarbonate, polystyrene, polyethylene, polypropylene, and polymethylmethacrylate.

23. (Amended) An analyte binding array for harvesting analyte from a liquid sample, the array comprising a plurality of microscopic sorbent zones immobilized on a substrate, wherein a microscopic sorbent zone comprises an analyte binding partner, the analyte binding partner being present in an amount sufficient to substantially deplete the analyte from a sample and concentrate the analyte on the microscopic sorbent zone, the microscopic zone being [less than] from about 60 to about 500 μm in diameter and the sample containing about 10^5 to about 10^{10} molecules of analyte per 100 μl of the sample, wherein a volume of the sample is from 20 to 500 μl.

26. (Amended) A kit for use in a binding assay that senses analyte mass in a liquid sample of a defined volume, comprising an analyte binding array [according to claim 23,] and a container comprising labeled binding partner,

wherein the analyte binding array comprises a plurality of microscopic sorbent zones immobilized on a substrate, wherein a microscopic sorbent zone comprises an analyte binding partner, the analyte binding partner being present in excess relative to the analyte, so that any analyte present in the defined volume of the sample is substantially depleted from the sample and concentrated on the microscopic sorbent zone to form an analyte capture complex with the analyte binding partner, and

the labeled binding partner having a fluorescent label and being capable of binding to an analyte bound by an analyte binding partner.